Food sources and lipid retention of zooplankton in subarctic ponds

HEATHER L. MARIASH*, MATTEO CAZZANELLI[†], MARTIN J. KAINZ[‡]
AND MILLA RAUTIO*,[§]

Department of Environmental & Biological Science, University of Jyväskylä, Jyväskylä, Finland
[†]*Freshwater Biological Laboratory, University of Copenhagen, Hillerød, Denmark*
[‡]*Inter-university Centre for Aquatic Ecosystem Research, Dr. Carl Kupelwiesser Promenade 5, Lunz am See, Austria*
[§]*Département des sciences fondamentales & Centre for Northern Studies (CEN), Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada*

SUMMARY

1. Subarctic ponds are seasonal aquatic habitats subject to short summers but often have surprisingly numerous planktonic consumers relative to phytoplankton productivity. Because subarctic ponds have low pelagic productivity but a high biomass of benthic algae, we hypothesised that benthic mats provide a complementary and important food source for the zooplankton. To test this, we used a combination of fatty acid and stable isotope analyses to evaluate the nutritional content of benthic and pelagic food and their contributions to the diets of crustacean zooplankton in 10 Finnish subarctic ponds.

2. Benthic mats and seston differed significantly in total lipids, with seston (62.5 μg mg^{-1}) having approximately eight times higher total lipid concentrations than benthic mats (7.0 μg mg^{-1}). Moreover, the two potential food sources differed in their lipid quality, with benthic organic matter completely lacking some nutritionally important polyunsaturated fatty acids (PUFA), most notably docosahexaenoic acid and arachidonic acid.

3. Zooplankton had higher PUFA concentrations (27–67 μg mg^{-1}) than either of the food sources (mean benthic mats: 1.2 μg mg^{-1}; mean seston: 9.9 μg mg^{-1}), indicating that zooplankton metabolically regulate their accumulation of PUFA. In addition, when each pond was evaluated independently, the zooplankton was consistently more ^{13}C-depleted (δ ^{13}C −20 to −33‰) than seston (−23 to −29‰) or benthic (−15 to −27‰) food sources. In three ponds, a subset of the zooplankton (*Eudiaptomus graciloides*, *Bosmina* sp., *Daphnia* sp. and *Branchinecta paludosa*) showed evidence of feeding on both benthic and planktonic resources, whereas in most (seven out of 10) ponds the zooplankton appeared to feed primarily on plankton.

4. Our results indicate that pelagic primary production was consistently the principal food resource of most metazoans. While benthic mats were highly productive, they did not appear to be a major food source for zooplankton. The pond zooplankton, faced by strong seasonal food limitation, acquires particular dietary elements selectively.

Keywords: benthic–pelagic coupling, fatty acids, food web, stable isotopes

Introduction

The ecological importance of benthic-derived material to lake food webs is receiving increasing attention (Rautio & Vincent, 2006; Vander Zanden *et al.*, 2006;

Correspondence: Heather L. Mariash, Department of Environmental & Biological Science, University of Jyväskylä, P. O. Box 35, 40014 Jyväskylä, Finland. E-mail: heather.mariash@jyu.fi

Brett, Müller-Navarra & Persson, 2009). It has been argued that the trophic transfer of benthic-derived primary production is especially important in shallow, clear lakes where a combination of restricted depth and low concentrations of dissolved organic carbon (DOC) allows for high production of benthic material (Vadeboncoeur *et al.*, 2003, 2008; Rautio & Vincent, 2006; Karlsson *et al.*, 2009). Hence, it has been widely suggested that benthic mats, defined here as cohesive microbial layers of algae attached to the substratum, may be important in sustaining high summer zooplankton biomass in oligotrophic, high-latitude ponds (Hobbie, 1980; Rautio & Vincent, 2006; Karlsson *et al.*, 2009). However, direct empirical evidence for the consumption and retention of benthic carbon by pond zooplankton is scarce.

The application of stable isotope analysis (SIA) to food web ecology has improved our ability to identify the resources used by secondary planktonic consumers (e.g. Karlsson *et al.*, 2003). A SIA approach requires different food sources to have distinct isotopic signatures. These signatures (e.g. $\delta^{13}C$ or $\delta^{15}N$) can then be traced through the food web because there is limited isotopic fractionation of $\delta^{13}C$ signatures between resources and consumers (Post, 2002); however, there is an enrichment of $\delta^{15}N$ signatures by *c.* 2–4‰ with each trophic transfer (e.g. Post, 2002; Matthews & Mazumder, 2008; and references therein). This trophic enrichment allows consumers to be placed sequentially into the food chain. In addition to carbon, zooplankton require dietary omega-3 (ω3) and ω6 polyunsaturated fatty acids (PUFA), which are integral parts of their cell membranes, improving somatic growth and helping maintain cell fluidity (Pruitt, 1990). Cell fluidity and fast growth are especially important in these high-latitude ponds where short, cold summers prevail. Zooplankton depend strongly on dietary lipids (e.g. Brett & Müller-Navarra, 1997) because, like other animals, they lack Δ12 and Δ15 desaturases and therefore cannot synthesise ω3 or ω6 PUFA *de novo* (Cook & McMaster, 2002). For this reason, zooplankton are expected to retain dietary PUFA selectively (Kainz, Arts & Mazumder, 2004; Hessen & Leu, 2006). Because dietary sources, in particular algae from different habitats, often exhibit distinct PUFA compositions, PUFA can be used as biomarkers for food sources (Brett *et al.*, 2006). Thus, fatty acid (FA) analysis can be coupled with a stable isotope approach to provide more detailed information on lacustrine food web structure (e.g. Perga *et al.*, 2006).

In high-latitude aquatic ecosystems, such as in subarctic Finland, organisms must cope with a short, ice-free growing season (July to September), low temperature (4–15 °C) and oligotrophy. Each of these environmental constraints increases with altitude, especially above the tree line where terrestrial subsidies are slight. Short summers and high zooplankton population densities imply intense competition for resources during the ice-free months (Van Geest *et al.*, 2007). The primary food source for zooplankton is pelagic algae but, by late summer, zooplankton communities have often exhausted these pelagic sources (Hessen & Leu, 2006). Consumers can cope with environmental constraints and competition either behaviourally by seeking alternative, lower-quality food resources or physiologically by sequestering higher-quality nutrient constituents, like PUFA. High primary benthic productivity in high-latitude ponds is known to make a significant contribution to whole-lake autochthonous production (Vadeboncoeur *et al.*, 2003, 2008) and could serve as a dietary complement to resource-limited zooplankton communities during late summer (Hansson & Tranvik, 2003; Rautio & Vincent, 2006; Karlsson *et al.*, 2009).

We designed a field study to evaluate the dietary role of pelagic and benthic food sources for zooplankton in oligotrophic, high-latitude ponds. We sampled three fractions of the seston, benthic mats and zooplankton in 10 subarctic ponds in northern Finland and analysed food availability and the biochemical quality of pelagic and benthic primary producers. In addition, we examined the relationship between these resources and the lipid composition of the five most prevalent metazoan groups in late summer. Previous experimental studies have shown that zooplankton can access benthic resources (Rautio & Vincent, 2006); however, no previous studies have quantified the quality of benthic mats as food. To our knowledge, this is the first field study to assess the dietary relevance of benthic mats to pond zooplankton. We hypothesised that zooplankton obtain part of their somatic needs from the benthic primary production and tested this using a combination of stable isotope and FA analyses. In addition, we hypothesised that, to compensate for the shorter growing season and lower temperatures at higher altitude, the standing stock of both food sources would have higher quality (in terms

of PUFA), which would be reflected in the primary consumers.

Methods

Samples were taken during August 2008 from 10 ponds in north-western Finland (69°04′N, 22°45′E). The study area is located within the Scandinavian mountain chain situated in the subarctic region, typically having only three to four ice-free months with a mean summer (June–August) air temperature of 9 °C. A characteristic feature of the area is the tree line of birch (*Betula pubescens* (Ehrh) subsp *czerepanovii* (Orlova), Hämet-Ahti) that approximately follows the 600-m contour. Of the 10 ponds sampled, four were below the tree line and six were above (Table 1). Temperature, conductivity and pH were measured from the upper metre of water in the centre of each pond once (YSI 63 meter; Yellow Springs Instruments, Yellow Springs, OH, U.S.A.). Water temperature was closely related to daily air temperature; only the deep 7-m pond was stratified (Lampo, pond 8). Water samples for DOC were filtered using 0.2-μm pore size cellulose acetate filter and analysed on a Shimadzu TOC-Vcph at the North Ostrobothnia Regional Environmental Centre, Finland.

Seston and benthic potential food sources were gathered from each pond. Bulk water samples (5–10 L) from the upper metre in the middle of the pond were strained through a 50-μm mesh to remove zooplankton while retaining the potentially edible particles (Kainz *et al.*, 2009). Seston was then isolated on pre-weighed, pre-combusted (400 °C for 2 h) glass fibre filters (Whatman GF/F, 0.7-μm pore size), from c. 3–5 L, depending on the particulate organic matter (POM) content of the pond. To have two additional fractions of the available resources within the POM, an additional 3–18 L of pond water was passed through a tangential flow filtration device (Millipore Pellicon 2 cassette filter; Millipore, Billerica, MA, U.S.A.); 1 L of the permeate (<0.2 μm) was retained while the back-wash was a concentrated sample of the original volume that did not pass through the filter (0.2–0.7 μm). The latter contained the bacterial fraction while the former was the DOC fraction of the seston; both were later freeze-dried and analysed for FA. Benthic cores were taken using a cylinder (diameter 5 cm and length 40 cm) that was hand-pushed into the sediment and closed with airtight caps at both ends before lifting the undisturbed sediment core from the pond. The cores were taken from three locations around the perimeter (20–60 cm depth) and at the centre of the ponds depending on the distribution of rock (epilithic) and sediment (epipelic) algae. The surface layer of each core was carefully sub-sampled using an 8-mm-diameter cut-off syringe (thickness 1 mm) to attain an undisturbed pellet of algae from the sediment–water interface. When epilithic algae were present, algae were scraped off from a fixed area (1–0.5 cm^2) using a scalpel from rocks found along the pond's perimeter. Both sample types were pooled in the data analysis to represent a single benthic source.

To assess general biomass and productivity of seston and benthic algae, we measured chlorophyll-*a* (chl-*a*) and the rate of photosynthesis. For seston chl-*a*, each water sample (0.5–1 L) was filtered onto a GF/F

Table 1 Physical and chemical characteristics of the study ponds located below and above the tree line. Numbers in parenthesis refer to pond coding in Rautio (2001)

	Pond no	Altitude (m)	Depth (m)	Area (m^2)	Temp (°C)	pH	Conductivity (μs cm^{-1})	Dissolved organic carbon (mg L^{-1})
Below tree line								
Masi (4)	1	500	1.3	118	8.9	7.2	35.1	2.9
Boat (3)	2	500	1.5	471	12.5	6.9	16.9	7.3
Big rock	3	527	0.5	47	15.2	4.9	21.0	6.0
Happy	4	531	1.0	471	11.6	6.8	20.1	6.4
Above tree line								
North Malla	5	545	3.0	11775	11.1	7.6	22.7	3.2
South Malla	6	599	2.5	7850	12.1	7.8	21.7	2.5
Reindeer (11)	7	710	1.5	1178	9.7	8.1	22.2	3.4
Lampo (15)	8	858	7.5	79	11.7	7.0	3.8	1.7
Tuono (14)	9	873	1.0	550	9.4	6.5	4.2	2.8
Straca	10	1000	0.5	47	4.9	7.1	9.2	1.2

filter and stored at −80 °C. For the analysis, the thawed sample was solubilised in 5 mL of 90% ethanol, placed in a water bath (75 °C, 5 mins), cooled and then analysed using spectrofluorometry (Yentsch & Menzel, 1963). For benthic chl-*a*, triplicates of benthic particles were freeze-dried prior to analysis to enhance extraction (Hansson, 1988) and were then analysed by spectrophotometry (Nusch, 1980). To estimate the algal proportion from the bulk seston and benthic mats from each site, we converted the chl-*a* concentration with a C/chl-*a* value of 40 : 1, a value obtained from a late summer phytoplankton community survey of Lake Saanajärvi in the vicinity of the sampled ponds (Rautio, Mariash & Forsström, 2011). The carbon values were divided by the concentration of particulate organic carbon (POC) to obtain the proportional algal contribution.

Primary productivity was measured *in situ* using the ^{14}C-bicarbonate protocol described by Rautio & Vincent (2006). Triplicates of pre-filtered (<50 μm) pond water (20 mL) were spiked with ^{14}C-bicarbonate (specific activity 80 μC mL^{-1}) and were incubated using five different light levels (0, 6, 25, 60 and 100% of total solar radiation) to obtain an estimate of the potential maximum pelagic photosynthetic rate (P_{max}) of the community. Owing to their heterogeneity, benthic algae were analysed for five replicates but for only three light levels (0, 25 and 100%) that were used to calculate the maximum productivity of the community (P_{max}). Filtered (<0.7 μm) pond water (20 mL) was used to suspend the benthic pellets. Each measurement included two dark bottles for both seston and benthic particles. Incubations were conducted outside under natural light in a 10 °C lake water bath for 2 h and were terminated by filtering the sample onto GF/F filters and freezing. Radioactivity was later determined with a liquid scintillation counter (RackBeta; LKB Wallac, Turku, Finland). Dissolved inorganic carbon titrations with 0.02 N NH_2SO_4 were made to determine carbon availability for photosynthesis in each pond.

Samples of seston and benthic organic matter were prepared for C and N stable isotope analyses to identify trophic links between zooplankton and different potential dietary sources. To estimate food quality, we quantified FA from both resource pools by taking the dry mass (DM) of benthic pellets and from the difference of seston filters to determine the fatty acid methyl ester (FAME) concentration (μg mg^{-1}

DM). In all localities, six replicates of each resource were taken, seston and benthic food sources were collected in the same way as described earlier, and then freeze-dried, of which three samples were stored at −20 °C for SIA and the remaining three were stored at −80 °C for FA analysis.

Zooplankton were collected using a 210-μm mesh hand net, sweeping the majority of the pond area. In addition, vertical tows (50-μm mesh net) were included for ponds more than 2 m deep and were later sieved through a 250-μm mesh to retain only the adults. Samples were stored in bottles, half of which were shock-frozen on dry ice in the field to minimise the change in FA composition. The other zooplankton were kept alive and stored at 4 °C overnight to allow for gut evacuation. The most abundant species (copepods: *Eudiaptomus graciloides* Lilljeborg 1888, cladocerans: *Bosmina* sp., *Daphnia* sp.; anostracans: *Polyartemia forcipata* Fischer 1851, *Branchinecta paludosa* Müller 1788) were then sorted, counted and compiled into three replicate Eppendorf tubes and kept in the freezer until SIA. Similarly, from the shock-frozen zooplankton, the most abundant species were handsorted and retained in triplicates for FA analysis. The number of individuals per replicate varied depending on size and taxon: Anostraca (three-15 individuals), *Daphnia* (20–100), *Eudiaptomus* (60–100) and *Bosmina* (200–500), aiming for a total DM > 0.5 mg.

Zooplankton have a greater lipid content than their food. If not corrected for, these lipids further deplete consumer δ^{13}C values (Syväranta & Rautio, 2010). Lipids were therefore removed from the zooplankton before SIA using a 1-mL wash of chloroform/methanol (2 : 1 v/v), slowly shaken overnight before removing the lipids. The remaining lipid-extracted zooplankton samples were set to dry before being weighed.

Dried seston filters, benthic pellets and zooplankton samples were analysed for δ^{13}C and δ^{15}N signatures using a FlashEA 1112 elemental analyser (Thermo Fisher Scientific Corporation, Waltham, MA, U.S.A.) coupled to a Thermo Finnigan DELTAplus Advantage mass spectrometer. Pike (*Esox lucius*, L.) white muscle tissue and potato leaves (*Solanum tuberosum*, L.) were used as internal working standards for zooplankton and for seston and benthic mats, respectively. Standards were inserted in each run after every five samples. Stable isotope ratios are expressed as delta values (δ^{13}C or δ^{15}N) relative to the international

standards for carbon (Vienna PeeDee belemnite) and nitrogen (atmospheric nitrogen). Internal precision for standards was usually <0.3‰ for both C and N in each run.

Lipids were analysed as described by Heissenberger, Watzke & Kainz (2010). In brief, freeze-dried and homogenised samples (0.5–1.5 mg) were extracted using chloroform–methanol. Fatty acids (C14-C24) were analysed as FAME after derivatisation using toluene and H_2SO_4–methanol. FAME were identified using a gas chromatograph with a flame ionisation detector (TRACE GC-FID THERMO™), equipped with a temperature-programmable injector and an autosampler. A Supelco™ SP-2560 column (100 m, 25 mm i.d., 0.2 μm film thickness) was used for FAME separation. Fatty acid concentrations were calculated using calibration curves based on known standard concentrations. For the quantified FA data, only concentrations >0.5 μg mg^{-1} DM were included.

Because earlier work has shown that ponds above and below the tree line differ significantly in their physicochemical characteristics and species composition (Rautio, 2001), we grouped the ponds into those lying above ($n = 6$) and below ($n = 4$) the tree line to examine the effect of altitude on resource quality.

Results

All of the ponds in this study fell within expected ranges for oligotrophic, tundra alpine waters in terms of chl-*a* and DOC contents (Kalff, 2001). These ponds also showed clear trends along the altitudinal gradient, with DOC (Table 1) decreasing linearly with altitude ($r^2 = 0.53$, d.f. = 9, $P = 0.007$) and conductivity values lower in the three highest ponds (<10 μS cm^{-1}, Table 1). In addition, benthic mats were found mainly below the tree line, while higher-altitude ponds had only thin epilithic algal cover. No other physical parameters were directly related to altitude or vegetation cover (Table 1).

Benthic mats had more chl-*a* and higher primary production than seston in all ponds (Table 2). Chl-*a* in benthic mats (0.7–41.8 mg m^{-2}) was an order of magnitude higher than in seston (0.3–2.8 mg m^{-2}). In five out of the 10 ponds, benthic mats contributed >90% of the potential maximum primary production. Benthic mats had similar proportions of algae compared with the bulk seston (3.5 ± 5% algal carbon in benthos and 2.6 ± 1% phytoplankton carbon in seston).

Carbon transfer and consumer acquisition

Stable isotope analysis results revealed two distinct food sources (Fig. 1). Mean δ^{13}C values of seston (−23 to −29‰) were significantly more depleted than those of benthic mats (−15 to −27‰; $P = 0.001$, d.f. = 1). In addition, benthic mats had lower δ^{15}N values than seston for eight out of the 10 ponds (Fig. 1a). The mean δ^{15}N value of all pond benthic mats was 0.2‰, while seston δ^{15}N values were, on average, 1.5 ‰.

Table 2 Chlorophyll-a (chl-*a*) concentrations (mg m^{-2}) and primary production as P_{max} (mg C m^{-2} h^{-1}) in the water column and in benthic mats in 10 Finnish subarctic ponds

	Pond no	Chl-*a*			Primary productivity (P_{max})			Dissolved inorganic carbon mg C m^{-3}
		Seston	Benthic	% Benthic	Seston	Benthic	% Benthic	
Below tree line								
Masi	1	1.1	8.8	88.9	1.0	40.2	97.6	2900
Boat	2	2.8	41.8	93.7	8.7	7.8	47.4	7300
Big rock	3	0.9	1.8	67.1	3.7	6.4	63.6	6000
Happy	4	0.8	4.8	85.9	4.9	43.2	89.9	6400
Above tree line								
North Malla	5	1.6	7.9	83.0	1.4	33.8	95.9	2500
South Malla	6	1.0	11.7	92.1	0.3	49.6	99.5	2500
Reindeer	7	1.2	8.8	88.4	–	3.3	–	3400
Lampo	8	2.3	2.8	54.8	–	–	–	1700
Tuono	9	1.0	3.7	78.6	0.2	11.6	98.4	2800
Straca	10	0.3	0.7	70.8	3.7	2.3	38.6	1200

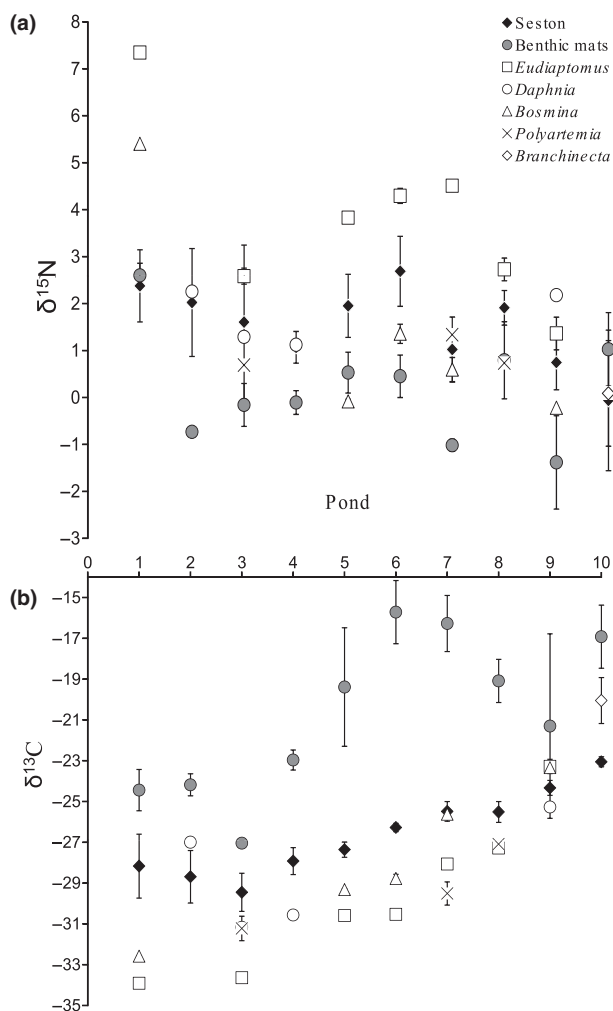**Fig. 1** Mean (±SE) isotope values of (a) $\delta^{15}N$ and (b) $\delta^{13}C$ for seston, benthic mats and zooplankton taxa for the ponds ordered by increasing altitude (1–10). Ponds 5–10 are above the tree line.

When each pond was evaluated independently, zooplankton $\delta^{13}C$ values (−20 to −33‰) were consistently more depleted than the respective seston values (Fig. 1b). Only four out of 19 populations had a $\delta^{13}C$ value higher than that of seston. The $\delta^{13}C$ values of *Daphnia* in pond 2 and *E. graciloides* and *Bosmina* in pond 9 were intermediate between seston and benthic values, suggesting consumption of both phytoplankton and benthic algae. In all other ponds where *Daphnia* (three ponds), *Bosmina* (four ponds) and *E graciloides* (five ponds) were present, their $\delta^{13}C$ values were lower than the respective seston value, indicating little to no consumption of benthic algae. Most zooplankton had higher $\delta^{15}N$ values than either

potential food source (Fig. 1a), with the exception of *Bosmina* sp., which generally had lower $\delta^{15}N$ values than seston. The enrichment in the zooplankton $\delta^{15}N$ values was especially pronounced in *E. graciloides*, which had a higher $\delta^{15}N$ value than both food sources (by up to 4.9‰ from benthic values).

Fatty acids and consumer retention

Analysis of the FA composition revealed distinct differences in resource quality between benthic and pelagic sources. Seston contained eight times more total lipids than benthic mats per unit DM. PUFA were consistently lower in benthic mats (1.2 ± 0.7 $\mu g\ mg^{-1}$ DM) than in seston (9.9 ± 5.7 $\mu g\ mg^{-1}$ DM). Notably, the PUFA docosahexaenoic acid (DHA; 22:6ω3) and arachidonic acid (ARA; 20:4ω6) were entirely absent from the benthic mats, while present in the seston of each pond. From the smaller-size fractions of the seston, the DOC and bacterial fraction contained no PUFA or any ω3 FA (Table 3). In the bacteria fraction, the FA found were mostly non-source specific (e.g. C16:0). Zooplankton FA concentrations differed when compared with their resources. From the suite of PUFA analysed, zooplankton had higher concentrations of the following PUFA than their dietary resources: linoleic acid (LIN; 18:2ω6), α-linolenic acid (ALA; 18:3ω3), stearidonic acid (SDA; 18:4ω3), ARA, eicosapentaenoic acid (EPA; 20:5ω3) and DHA (Table 4). The only monounsaturated fatty acid found at higher concentrations in zooplankton than in seston or benthic mats was vaccenic acid (18:1ω7). The observed high PUFA retention made PUFA the most prevalent group of FA in zooplankton, closely followed by saturated fatty acids (SAFA; Table 4).

Comparing the difference in FA concentrations between zooplankton and their resources provides a means of quantifying consumer lipid retention. Thus, we calculated PUFA retention ratios, by dividing PUFA concentrations of zooplankton by PUFA concentrations of their potential diet [i.e. $(PUFA)_{zooplankton}/(PUFA)_{diet}$], to assess the trophic relationship between dietary PUFA supply and PUFA retention in the consumer. When comparing retention ratios between zooplankton and seston or benthic sources, all zooplankton had the smallest PUFA retention ratios from seston (by a multiple of 10), suggesting that seston supplies more PUFA to herbivorous consumers than does the benthic mat.

Table 3 Mean concentration (μg mg^{-1} dry mass) of individual fatty acid food resources: DOC (<0.2 μm), the bacterial fraction (0.7–0.2 μm), benthic organic matter and seston (>0.7 μm) from 10 ponds below and above the tree line in subarctic Finland. Only lipids accounting for >0.5 μg mg^{-1} are shown. Mean total fatty acid percentages included. Below the tree line $n = 4$, above $n = 6$

Fatty acids	DOC <0.2 μm				Bacteria (0.2–0.7 μm)				Benthic mats				Seston			
	Below	Above ($n = 5$)	Mean	%	Below ($n = 3$)	Above ($n = 5$)	Mean	%	Below	Above	Mean	%	Below	Above	Mean	%
SAFA	0.3	0.8	0.6	58	1.1	1.7	1.5	48	3.2	2.6	2.9	41	34.2	36.8	35.8	57
MUFA	0.2	0.3	0.2	24	1.2	1.5	1.4	45	2.4	1.8	2.0	29	17.2	16.9	17	27
PUFA	0.0	0.0	0.0	0	0.0	0.0	0.0	0	1.2	1.1	1.2	17	8.9	10.6	9.9	16
20–22C PUFA	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0.9	0.3	0.5	7	4.6	3.3	3.8	6
LIN	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0	0.2	0.1	2	2.7	2.9	2.9	5
ALA	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0.1	0.1	0.1	1	1.7	2.8	2.3	4
SDA	–	–	–	–	–	–	–	–	3	6.5	3.8	0	5.2	7.7	4.3	1
ARA	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0	0	0.0	0	0.5	0.3	0.4	1
EPA	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0	0.2	0.1	2	1.2	1.6	1.4	2
DHA	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0	0	0.0	0	1.1	0.8	0.9	1
ω-6	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0	0	0.0	0	0	0	0	0
ω-3	0.0	0.0	0.0	0	0.0	0.0	0.0	0	0.6	0.5	0.6	8	4	5.3	4.8	8
Total lipids	0.7	1.3	1.0	100	2.5	3.4	3.1	100	7.4	6.7	7.0	100	55.9	66.8	62.5	100

ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; DOC, dissolved organic carbon; EPA, eicosapentaenoic acid; LIN, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; SDA, stearidonic acid.

Table 4. Mean concentration (μg mg^{-1} dry mass) of individual fatty acids in zooplankton from 10 ponds below and above the tree line in subarctic Finland. Only lipids accounting for >0.5 μg mg^{-1} are shown. Mean total fatty acid percentages included for the zooplankton

Fatty acids	*Eudiaptomus*				*Daphnia*		*Bosmina*				*Polyartemia*				*Branchinecta*		Zooplankton	
	Below	Above	Mean	%	Below	%	Below	Above	Mean	%	Below	Above	Mean	%	Above	%	Bulk	%
SAFA	50.4	62.5	56.4	36	39.6	39	39.3	40	39.9	41	20.5	10.1	18.3	25	13.6	23	40.6	36
MUFA	22.0	23.1	22.5	14	29.4	29	24.4	26	25.8	26	22.0	9.8	18.3	25	18.1	30	24.8	22
PUFA	55.2	78.7	67.0	43	29.4	29	23.8	32	30.1	31	33.5	19.0	32.6	45	26.8	44	42.7	38
20–22C PUFA	33.2	49.5	41.4	26	16.9	17	14.8	18	17.6	18	20.5	13.4	22.1	31	15.7	26	26.1	23
LIN	7.0	10.4	8.7	6	5.4	5	6.0	5	5.3	5	4.9	1.5	3.6	5	4.8	8	6.4	6
ALA	9.1	10.6	9.9	6	5.1	5	3.0	5	4.6	5	7.2	1.8	5.1	7	4.9	8	6.6	6
SDA	8.6	4.3	3.2	2	3.8	4	2.1	0	8.4	6	1.8	0.4	4.6	3	2.8	4	2.4	4
ARA	3.0	3.4	3.2	2	4.3	4	5.2	6	5.8	6	3.2	1.1	2.2	3	2.7	4	4.1	4
EPA	10.3	16.2	13.3	8	11.4	11	8.7	11	10.6	11	12.9	7.6	13.0	18	10.7	18	12.5	11
DHA	17.2	25.8	21.5	14	0.2	0	0.9	1	1.0	1	3.3	3.8	5.0	7	1.5	2	7.8	7
ω-6	1.3	1.3	1.3	1	0.4	0	0.0	1	0.4	0	0.5	0.0	0.1	0	0.0	0	0.6	1
ω-3	37.6	53.9	45.8	29	17.0	17	12.6	18	16.6	17	23.8	13.4	23.4	32	17.3	28	27.4	24
Total lipids	133.8	179.6	156.7	100	101.3	100	87.6	100	98.2	100	78.9	40.8	72.3	100	60.6	100	113.4	100

ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LIN, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; SDA, stearidonic acid.

Fatty acid retention varied significantly between zooplankton taxa (Fig. 2). While all taxa had higher PUFA concentrations than either benthic or seston resources, cladocerans (*Daphnia* and *Bosmina* sp.) retained lower concentrations of DHA relative to their pond-specific seston source (Fig. 2). Of all PUFA,
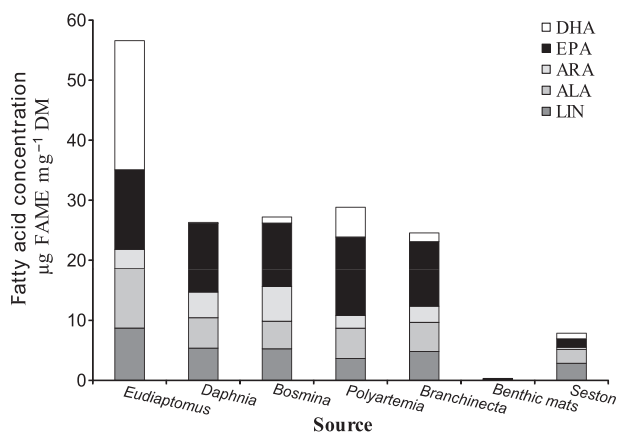
Fig. 2 Mean concentrations of essential fatty acid in *Eudiaptomus graciloides*, *Daphnia* sp., *Bosmina* sp., *Polyartemia forcipata* and *Branchinecta paludosa* and in their two potential food sources, benthic mats and seston. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid; ALA, α-linolenic acid; LIN, linoleic acid. Benthic mats contained 0.2 µg mg^{-1} DM (the detection limit) only of LIN, ALA and EPA.

cladocerans retained EPA most efficiently (11 ± 2.4 µg mg^{-1} DM). For *E. graciloides*, a third of all their PUFA was DHA (21.5 ± 2.6 µg mg^{-1} DM; Fig. 2), which is substantially higher than the average zooplankton DHA values excluding *E. graciloides* (mean 1.9 ± 2.1 µg mg^{-1} DM).

Elevation gradient

The altitudinal gradient contributed to differences in δ^{13}C values of seston, which increased with increasing altitude ($r^2 = 0.68$, $P = 0.002$, d.f. = 9). This trend was mirrored by zooplankton δ^{13}C values that also increased with altitude ($r^2 = 0.68$, $P < 0.001$, d.f. = 17).

All PUFA, except SDA, were absent from benthic mats below the tree line. In mats above the tree line, SDA doubled and trace amounts of ALA, LIN and EPA were present, although, nevertheless, the overall FA concentration was very low (Fig. 3a). For seston, there were only slight increases in PUFA concentrations above the tree line, although ARA and DHA decreased (Fig 3b). Altitude, specifically the tree line, affected FA concentrations of benthic mats more than it did for seston (Table 3).

The taxonomic composition of zooplankton and their FA retention also changed with altitude. Cladocerans were primarily found in ponds below the tree line, and *B. paludosa* only above the tree line. *Eudiaptomus graciloides*, which was the only taxon found in ponds both above and below the tree line, contained significantly higher total lipid concentrations (Fig. 3c, Table 4) in ponds above the tree line (Wilcoxon signed-rank test: $P < 0.05$, d.f. = 5). In
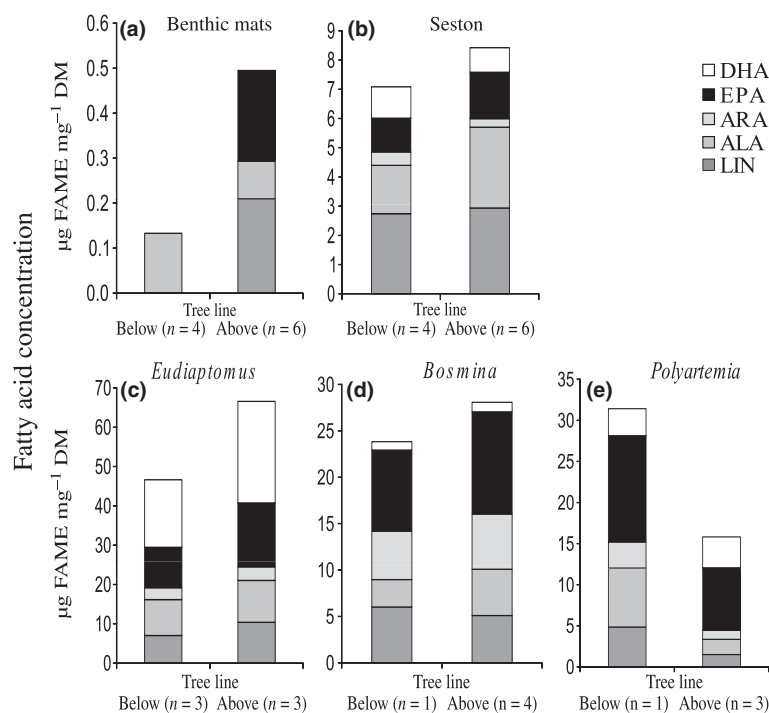
Fig. 3 Mean essential fatty acid concentrations in (a) benthic mats, (b) seston and in three prevalent zooplankton taxa: (c) *Eudiaptomus* (d) *Bosmina* and (e) *Polyartemia* from ponds above (n = 6) and below (n = 4) the tree line. Note the different vertical scales. Docosahexaenoic acid: 22:6ω3; eicosapentaenoic acid: 20:5ω3; arachidonic acid: 20:4ω6; α-linolenic acid: 18:3ω3; linoleic acid: 18:2ω6.

retained at higher concentrations in all the consumers compared with the food sources was 18:1ω7, a FA found in heterotrophic bacteria (Vainshtein, Hippe & Kroppenstedt, 1992). For physiological reasons that are not clear, the retention of 18:1ω7 in zooplankton suggests that the consumers preferentially retained this FA over others detected in bacteria. It is likely that the bacteria of subarctic ponds contribute some energy to zooplankton, as has been shown with SIA and direct feeding experiments for waterbodies ranging from humic lakes (Taipale *et al.*, 2008) to subarctic ponds (Rautio & Vincent, 2006). However, it is unlikely that bacteria supply dietary PUFA that are required for cell membranes and somatic growth of zooplankton (Brett *et al.*, 2009). The lack of PUFA in the bacterial and DOC fraction of the seston indicates that the FA found in zooplankton are derived from POM (0.7–50 μm).

Since FA composition differed among zooplankton species, the potentially growth-limiting substance may have also differed from species to species (Persson & Vrede, 2006; Smyntek *et al.*, 2008). Both those studies showed that cladocerans preferentially retain EPA, while copepods accumulate more DHA than EPA. Similarly in our study, the main taxon-specific difference in FA retention was between *Daphnia* and *Eudiaptomus*, showing that *Daphnia* did not retain DHA, yet contained nearly twofold higher concentrations of SAFA, EPA and ARA than in Persson & Vrede's (2006) study of high-latitude ponds. Conversely, *E. graciloides* contained 20 times more DHA than the concentration in the seston. In addition, *Eudiaptomus* had high concentrations of EPA, but to a lesser extent than *Daphnia*. This preference for specific FA is based on differences in life history strategies and evolved physiological adaptations (Brett *et al.*, 2006; Persson & Vrede, 2006). For instance, species have particular adaptations to cope with certain environmental conditions (e.g. cold), which is the impetus to regulate metabolically different concentrations of specific FA (Smyntek *et al.*, 2008).

The differences in FA profiles between seston and benthic mats may be attributed to differences in FA composition of algal populations. Flagellates, including Chrysophyta, Cryptophyta and Dinophyta, dominate the phytoplankton of northern Finland (Forsström, Sorvari & Korhola, 2005). However, benthic mats are known to be composed primarily of Cyanobacteria, Chlorophyta and Dinophyta (L. Forsström, pers. comm), which are also frequently found in benthic mats of other regions (Bonilla *et al.*, 2009). Cyanobacteria synthesise very little EPA or DHA (Brett *et al.*, 2006, 2009). In addition, our FA and δ^{15}N value results from benthic mats are both consistent with the presence of nitrogen-fixing cyanobacteria; neither DHA nor ARA was detected in the benthic mats, and near-zero δ^{15}N values of the benthos are consistent with values for nitrogen-fixing cyanobacteria (Vincent, 2000). Zooplankton are known to discriminate against cyanobacteria (Sarnelle, Gustaffsson & Hansson, 2010). Diatoms and cryptophytes readily synthesise PUFA, whereas green algae and cyanobacteria produce only shorter-chain PUFA (Brett *et al.*, 2009).

The amount of accumulated benthic algae varied between ponds; highly productive, thick filamentous mats were mainly found in ponds below the tree line. Despite their high biomass, there was no indication of more consumer reliance on their benthic mats than in those above the tree line. Rather, zooplankton δ^{13}C values reflected changes in seston rather than in the benthic mats along the altitudinal gradient. Similarly, Rautio & Vincent (2007) showed that, in Canadian subarctic and arctic ponds, zooplankton often had a δ^{13}C value closer to the seston than to the benthos, although feeding on both food resources was more common in the communities they studied.

As we hypothesised, both food resources have higher-quality FA at higher altitudes. PUFA concentration is inversely correlated with trophic status (Müller-Narvarra *et al.*, 2004), which implies that phytoplankton will have more PUFA at higher altitudes, as is evident in our study. Increasing altitude accentuates seasonal constraints, making it necessary, also for consumers, to maximise their retention of high-quality carbon. Coping with seasonal constraints is directly related to the need to maintain fluidity in cold temperatures (Schlechtriema, Arts & Zellmer, 2006) and maintain efficient growth and reproduction in a shorter growing season, which requires EPA (Müller-Narvarra *et al.*, 2000). Therefore, the higher PUFA concentration found in zooplankton at higher altitudes is partly due to the food sources having higher quality and partly from consumers' selective retention.

Zooplankton have evolved the ability to balance their individual metabolic needs in harsh environ-

mental conditions for optimal success. Specifically, this could involve selective feeding (i.e. whether they access benthic resources), an example of behavioural plasticity. Additionally, consumers can metabolically select how the energy acquired is sequestered within the soma (Müller-Navarra, 2008; Smyntek *et al.*, 2008). This metabolic plasticity could be the cause of the higher zooplankton PUFA concentrations above the tree line. Higher concentrations of EPA, DHA and SDA relative to resources in *E. graciloides* demonstrated preferential retention of these PUFA, and this was most pronounced in ponds above the tree line. These biochemical adaptations for selecting and retaining desired FA offer a mechanism by which consumers can enhance their survival. Further understanding of energy acquisition and selective PUFA retention is needed to follow more accurately the dynamics of subarctic food web structure and function. For example, consumer competitive relationships and species composition are affected by resource quality, particularly when the acquisition of nutritional requirements has consequences for somatic growth and reproductive success of consumers (Persson & Vrede, 2006).

In conclusion, our field study has shown that the phytoplankton in the seston probably provides zooplankton in subarctic ponds with essential dietary lipids in sufficient quantities to meet demand, even in late summer. The pelagic resource contained the dietary PUFA required in greater concentrations than benthic resources. In addition, higher retention of PUFA and ω3 FA in *E. graciloides* in ponds above the tree line provides field evidence of metabolic regulation of these physiologically important lipids.

References

Bertilsson S., Hansson L.-A., Graneli W. & Philibert A. (2003) Size-selective predation on pelagic microorganisms in Arctic freshwaters. *Journal of Plankton Research*, **25**, 621–631.

Bonilla S., Rautio M. & Vincent W.F. (2009) Phytoplankton and phytobenthos pigment strategies: implications for algal survival in the changing Arctic. *Polar Biology*, **32**, 1293–1303.

Brett M.T. & Müller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology*, **38**, 483–499.

Brett M.T., Müller-Navarra D.C., Ballantyne A.P., Ravet J.L. & Goldman C.R. (2006) *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography*, **51**, 2428–2437.

Brett M.T., Müller-Navarra D.C. & Persson J. (2009) Chapter 6: Crustacean Zooplankton fatty acid composition. In: *Lipids in Aquatic Ecosystems* (Eds M. Art, M.T. Brett & M.J. Kainz), pp. 115–146. Springer Dordrecht Heidelberg, New York.

Cook H.W. & McMaster C.R. (2002) Chapter 7: fatty acid desaturation and chain elongation in eukaryotes. In: *Biochemistry of Lipid, Lipoproteins and Membranes* (Eds D.E. Vance & J.E. Vance), pp. 181–204. Elsevier, Amsterdam.

Forsström L., Sorvari S. & Korhola A. (2005) The role of the environmental factors in controlling chrysophyte species distribution and biomass structure in Finnish Lapland subarctic lakes. *Nova Hedwigia*, **128**, 179–187.

France R.L. (1995) Carbon-13 enrichment in benthic compared to pelagic algae: food web implications. *Marine Ecology Progress Series*, **124**, 307–312.

Hansson L. (1988) Chlorophyll a determination of periphyton on sediments: identification of problems and recommendation of method. *Freshwater Biology*, **20**, 347–352.

Hansson L. & Tranvik L.J. (2003) Foodwebs in sub-Antarctic lakes: a stable isotope approach. *Polar Biology*, **26**, 783–788.

Hecky R.E. & Hesslein R.H. (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *The North American Benthological Society*, **14**, 631–653.

Heissenberger M., Watzke J. & Kainz M.J. (2010) Effect of nutrition on fatty acid profiles of riverine, lacustrine

and aquaculture-raised salmonids of pre-alpine habitats. *Hydrobiologia*, **650**, 243–254.

Hessen D. & Leu E. (2006) Trophic transfer and trophic modification of fatty acids in high Arctic lakes. *Freshwater Biology*, **51**, 1987–1998.

Hobbie J.E. (1980) *Limnology of Tundra Ponds*. Dowden, Hutcgubsin Ross, Inc., Stroudsburg, PA, USA.

Kainz M.J., Arts M.T. & Mazumder A. (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. *Limnology and Oceanography*, **49**, 1784–1793.

Kainz M., Perga M., Arts M.T. & Mazumder A. (2009) Essential fatty acid concentrations of different seston sizes and zooplankton: a field study of monomictic coastal lakes. *Journal of Planktonic Reseach*, **31**, 635–645.

Kalff J. (2001) *Limnology: Inland Water Ecosystems*, pp. 119. Prentice-Hall, Inc, Upper Saddle River, NJ.

Kankaala P., Taipale S., Grey J., Sonninen E., Arvola L. & Jones R. (2006) Experimental d13C evidence for a contribution of methane to pelagic food webs in lakes. *Limnology and Oceanography*, **51**, 2821–2827.

Karlsson J., Jonsson A., Meili M. & Jansson M. (2003) Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography*, **48**, 269–276.

Karlsson J., Byström P., Ask J., Ask P., Persson L. & Jansson M. (2009) Light limitation of nutrient-poor lake ecosystems. *Nature*, **460**, 506–509.

Marty J. & Planas D. (2008) Comparison of methods to determine algal δ13C in freshwater. *Limnology and Oceanography: Methods*, **6**, 51–63.

Matthews B. & Mazumder A. (2008) Detecting trophic-level variation in consumer assemblages. *Freshwater Biology*, **53**, 1942–1953.

Müller-Narvarra D.C., Brett M.T., Liston A.M. & Goldman C.R. (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, **403**, 74–77.

Müller-Narvarra D.C., Brett M.T., Park S., Chandra S., Ballantyne A.P., Zorita E. *et al.* (2004) Unsaturated fatty acid content in seston and trophodynamic coupling in lakes. *Nature*, **427**, 69–72.

Müller-Navarra D.C. (2008) Food web paradigms: the biochemical view on trophic interactions. *International Review of Hydrobiology*, **93**, 489–505.

Nusch E.A. (1980) Comparison of different methods for chlorophyll and phaeopigments determination. *Archives for Hydrobiologia*, **14**, 14–35.

Perga M., Kainz M., Matthews B. & Mazumder A. (2006) Carbon pathways to zooplankton: insights from the combined use of stable isotope and fatty acid biomarkers. *Freshwater Biology*, **51**, 2041–2051.

Persson J. & Vrede T. (2006) Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. *Freshwater Biology*, **51**, 887–900.

Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.

Pruitt N.L. (1990) Adaptations to temperature in the cellular membranes of custacea- membrane structure and metabolism. *Journal of Thermal Biology*, **15**, 1–8.

Rautio M. (2001) Zooplankton assemblages related to environmental characteristics in tree line ponds in Finnish Lapland. *Arctic, Antarctic, and Alpine Research*, **33**, 289–298.

Rautio M. & Vincent W.F. (2006) Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. *Freshwater Biology*, **51**, 1038–1052.

Rautio M. & Vincent W.F. (2007) Isotopic analysis of the sources of organic carbon for zooplankton in shallow subarctic and arctic waters. *Ecography*, **30**, 77–87.

Rautio M., Mariash H. & Forsstöm L. (2011) Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake. *Limnology & Oceanography*, **54** (in press).

Sarnelle O., Gustaffsson S. & Hansson L. (2010) Effects of cyanobacteria on fitness components of the herbivore Daphnia. *Journal of Planktonic Reseach*, **32**, 471–477.

Schlechtriema C., Arts M.T. & Zellmer I.D. (2006) Effect of temperature on the fatty acid composition and temporal trajectories of fatty acids in fasting Daphnia *pulex* (Crustacea, Cladocera). *Lipids*, **41**, 397–400.

Smyntek P.M., Teece M.A., Schulz K.L. & Storch A.J. (2008) Taxonomic differences in the essential fatty acid composition of groups of freshwater zooplankton relate to reproductive demands and generation time. *Freshwater Biology*, **53**, 1768–1782.

Syväranta J. & Rautio M. (2010) Zooplankton, lipids and stable isotopes: importance of seasonal, latitudinal and taxonomic differences. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1721–1729.

Taipale S., Kankaala P., Tiirola M. & Jones R.I. (2008) Whole-lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet. *Ecology*, **89**, 463–474.

Vadeboncoeur Y., Jeppesen E., Vander Zanden J.M., Schierup H., Christofferson K. & Lodge D.M. (2003) From Greeland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography*, **48**, 1408–1418.

Vadeboncoeur Y., Peterson G., Vander Zanden J.M. & Kalff J. (2008) Benthic algal production across lake size

gradients: interaction among morphometry, nutrients, and light. *Ecology*, **89**, 2542–2552.

Vainshtein M., Hippe H. & Kroppenstedt R.M. (1992) Cellular fatty acid composition of Desulfovibrio species and its use in classification of sulfate-reducing bacteria. *Systematic and Applied Microbiology*, **15**, 554–566.

Van Geest G.J., Spierenburg P., Van Donk E. & Hessen D. (2007) Daphnia growth rates in arctic ponds: limitation by nutrients or carbon? *Polar Biology*, **30**, 235–242.

Vander Zanden J.M., Sudeep C., Park S.K., Vadeboncoeur Y. & Goldman C.R. (2006) Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. *Canadian Journal of Aquatic Sciences*, **63**, 2608–2620.

Vincent W.F. (2000) Cyanobacterial dominance in the polar regions. In: *Ecology of the Cyanobacteria: Their Diversity in Space and Time* (Eds B.A. Whitton & M. Potts), pp. 321–340. Kluwer Academic Publishers, Dordrecht, The Netherlands..

Yentsch C.S. & Menzel D.W. (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-sea Research*, **10**, 221–231.